



**Assay: N2P-RPP30 (100 reactions):  
SARS CoV-2 Delta and earlier Variant of Concern  
with RPP30 control,  
N-gene: 31-33 region  
PCR mastermix included  
(RUO). Research Use Only. Not for use in  
Diagnostic Procedures.**

**Kit contents:**

- Tube 1: 20X primers/probe specific for Delta and earlier VOCs.
  - Tube 2: 20X primers/probe specific for spliced human RPP30 mRNA.
  - Tube 3: 2X TaqMan mastermix.
  - Tube 4: 40X RT enzyme.
- (Tubes 3 and 4 are from Empirical Biosciences).

**Note: molecular biology grade water should be used to prepare the PCR reactions, which is NOT included in this kit.**

**INTRODUCTION**

The DNA Software assay N2P-RPP30 is a real-time reverse transcriptase polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from VOCs before Omicron (*i.e.*, original Wuhan strain, alpha, beta, gamma, or delta VOCs) with CalFluorRed610™ (a trademark of Biosearch Technologies, Inc). This kit is for research use only and should not be used for diagnostic procedures.

This kit is pre-validated with three standard synthetic RNA genomes from Twist Biosciences: SARS-CoV-2 Delta variant (Twist® Standard RNA #18), Omicron variant BA.1 (Twist® Standard RNA #48) and Omicron variant BA.2 (Twist® Standard RNA #50). The limit of detection (LOD) is below 20 copies/reaction for the Delta variant. The CalFluorRed610™ probe is specific to SARS-CoV-2 Delta variant (and the variants prior to Delta by sequence analysis) and no cross-reactivity was observed with the standard synthetic RNAs of SARS-CoV-2 Omicron variants (Twist® Standard RNA #48 and #50).

**CONTENTS**

A mix of primers/probe targeting the RNA region coding for N protein (amino acid region 31-33) in SARS-CoV-2 genome is provided in a tube as a 20X concentrated working solution. The fluorophore of the probe for Delta and earlier VOCs is CalFluorRed610™ and the quencher is BHQ-2™ (Black Hole Quencher, a trademark of Biosearch Technologies, Inc.). A mix of primers/probe targeting spliced human RPP30 mRNA is also provided in a tube (a 20X concentrated working solution) as a RT-PCR positive control for human samples. The fluorophore of the probe is HEX™ (Hexachloro-fluorescein, a trademark of Life Technologies, Inc), and the quencher is BHQ-1™. An alternative RNA positive control of PMMoV is also available for wastewater samples as a 20X concentrated working solution with HEX™ fluorophore. Other positive control(s) should be used in place of the human RPP30 mRNA or PMMoV primers/probe if the samples originate from other sources. Users are responsible to provide such alternative control(s).

The reverse transcriptase and TaqMan mastermix from Empirical Bioscience (items: RTqPCR-Kit-200) are included in this kit. This Empirical kit provided reproducible and reliable results in prevalidation experiments and is recommended for applications with the N2P-RPP30 kit. See EXPERIMENTAL for more details.

**KIT HANDLING AND CONTAMINATION**

The DNA Software Assay N2P-RPP30 is shipped with ice packs, and should be stored at -30 to -15°C. The kit should be kept on ice once thawed.

Any contamination should be avoided by using appropriate personal protective equipment (PPE), powder free gloves, aerosol barrier pipette tips, and a clean hood.

**EXPERIMENTAL**

Set up your reaction (20 µL) as follows on ice:

Component	Volume (µL)
TaqMan mastermix	10
RT enzyme	0.5
N2P primers/probe mix	1
RPP30 mRNA primers/probe mix	1
Sample	2
Water	5.5

**Note: The volume of water should be adjusted accordingly if the user's reaction preparation is different from the recommended preparation method.**

An RT-PCR protocol was used at DNA Software, Inc. for pre-validation on a Bio-Rad CFX96™ Real-Time System, with the following program:

Step	Thermocycling Protocol:
1	Incubate @ 50 °C for 5 minutes
2	Incubate @ 94 °C for 3 minutes
3	Incubate @ 94 °C for 5 seconds
4	Incubate @ 63 °C for 30 seconds
5	Plate Read
6	Go to Step 3, repeat 44x more
7	(optional) Incubate @63 °C for 3 minutes

## RESULT INTERPRETATION

After running the qPCR reaction, perform a regression analysis on the data to determine the quantification cycle, C<sub>q</sub>. (C<sub>q</sub> is preferred over Ct). Each fluorescence channel with a C<sub>q</sub> < 38 cycles and final RFU >200 is considered “positive” or “+” in the Table below.

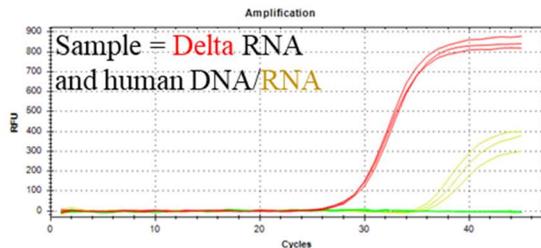
Delta and earlier VOCs (CalFluorRed610™)	RPP30 (HEX™)	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment.
–	+	The sample doesn't contain SARS-CoV-2 Delta or earlier VOC RNA.
+	–	The sample contains SARS-CoV-2 Delta or earlier VOC RNA. The sample may not contain spliced human RPP30 mRNA.
+	+	The sample contains SARS-CoV-2 Delta or earlier VOC RNA and spliced human RPP30 mRNA.

## PRE-VALIDATION DATA

Experiments were performed in triplicate using the experimental procedure given above, but with different samples added to each reaction. The samples used for the validation experiments contained 1×10<sup>5</sup> copies/reaction synthetic viral RNA obtained from Twist Biosciences as follows:

- SARS-CoV-2 Delta variant (Twist® Standard RNA #18)
- SARS-CoV-2 Omicron BA.1 variant (Twist® Standard RNA #48)
- SARS-CoV-2 Omicron BA.2 variant (Twist® Standard RNA #50)

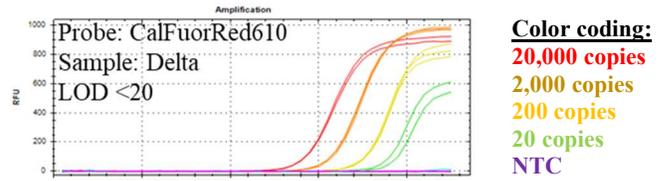
The samples also contained human brain RNA (1500 copies) from Roche and human genomic DNA (3100 copies) from Clontech. The RPP30 control primers and probe specifically reverse transcribe and amplify the human RPP30 mRNA and not genomic DNA (See DNAS Product insert about RPP30 RNA control for more information). The presence of the human genomic DNA in the reaction appears to have no effect on the amplification of SARS-CoV-2 RNA (data not shown). The results of these experiments are shown in **Figure 1** below:



**Figure 1:** Validation experiments of the N2P-RPP30 kit with single targets (given in text boxes for each panel) and human mRNA. The **CalFluorRed610** probe detects the Delta variant (Twist #18). The **HEX** probe detects spliced human RPP30 mRNA.

**Conclusion:** The data in **Figure 1** indicate that the N2P-RPP30 assay specifically detects the Delta variant of SARS CoV-2. Based on sequence analysis, it can be inferred that the N2P-RPP30 assay will also detect in the CalFluorRed Channel the reference SARS CoV-2 (*i.e.*, Wuhan strain) and other earlier VOCs such as alpha, beta, and gamma (data not shown).

Limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate (**Figure 2**). For the dilution series experiment, Delta template RNA was added. The results show a limit of detection (LOD) <20 copies/reaction.



**Figure 2:** Serial dilution experiment of the Delta variant shows that the N2P-RPP30 kit has LOD <20 molecules.

## CONTACT US

For further assistance, please contact DNA Software using the link: <https://www.dnasoftware.com/contact/>

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